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Original Article

Changes In the Levels of Serum Iron, Total Iron-Binding Capacity and Erythrocyte Sedimentation Rate and Association with Work-Related Exposure to Petrochemical Products in Petrol Station Attendants

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Abstract:
Background: Acute and chronic exposure to petrochemical products through inhalation or ingestion affects the health of individuals, with prolonged exposure likely to cause more adverse effects. Attendants at petrol stations in developing countries rarely use protective wear and are usually occupationally exposed to petrochemical gases. It is hypothesized that exposure to petrochemical gases may interfere with iron homeostasis.
Objective: This study examined the changes in the levels of serum iron, total iron binding capacity (TIBC) aand erythrocyte sedimentation rate (ESR) in relation to work-related exposure to petrochemical products in petrol station attendants.
Method: This cross-sectional study included 100 age and sex matched participants comprising 50 petrol station attendants and 50 control subjects. Venous blood samples were collected from the subjects and analyzed for serum iron, TIBC and ESR. The Statistical Package for Social Sciences version 25 was used for statistical analysis.
Results: The mean levels of serum iron and TIBC were significantly lower in petrol station attendants than in control participants. However, the ESR did not differ significantly between the two groups. The levels of serum iron and TIBC was decreased with longer work duration (> 12 months) than with 1- 6 months duration, whereas the ESR increased with longer duration of work-related exposure (>12 months) than with shorter durations of work-related exposure (7-12 months and 1-6 months respectively) ($p<0.05$). There was also a significant positive correlation, between work-related duration of exposure to petrochemical gas and ESR and a significant negative correlation between the work-related duration of exposure and TIBC ($P< 0.05$). Additionally, male petrol station attendants had significantly higher levels of serum iron than females, whereas the ESR was greater in females than in males. Additionally, male control participants had higher levels of serum iron and TIBC than females, while the ESR was greater in females than the in males ($P<0.05$).
Conclusion: Petrol station attendants had significantly lower levels of serum iron and TIBC than unexposed control. This may imply that occupational exposure to petrochemical products may affect iron homeostasis.

Keywords: Iron; Petrol Station Attendants; Inflammation; Erythrocyte Sedimentation Rate

Introduction

Chronic exposure to pollutants such as petroleum products via occupational and environmental sources constitutes health hazards because they compositionally contain benzene which is harmful and toxic to the body systems [1]. This effect is more in individuals who do not use protective shields whether they are domestically or occupationally exposed to petroleum products. Petroleum products are very volatile in nature and easily aerosolized into the atmosphere during dispensing at petroleum stations. Motorists and cyclists at the point of refueling their automobiles and other workers at petrol stations are at risk of exposure to these fumes and aerosols. However, petrol station attendants are exposed to more risk because of the extended length of time they spend and work at petrol stations daily. In developing countries such as Nigeria, there is no clear regulation to limit exposure to these harmful substances. Additionally, individuals such as petrol station attendants who are occupationally exposed to these toxic fumes from petroleum products observe little or no safety measures when handling these products [2]. These petrol station attendants work for an average of 8 - 10 hours daily and do not use protective shields such as face or nose masks or face shields to minimize exposure to these gases either out of ignorance or carelessness.

Petrochemical gases are a group of volatile organic compounds (VOCs), derived from petroleum or natural gas sources and are commonly used in the internal combustion of machines and various industries. Human health status is adversely affected by exposure to petrochemical gases through several sources that include inhalation of the fumes and ingestion [3]. Petrochemical gases as assessed by the US Agency for Toxic Substances and Disease Registry, are made up of over one hundred and fifty chemicals that includes varying amounts of xylene, benzene, ethyl benzene, toluene (collectively labeled BTEX) and trace quantities of lead as contaminants [4]. Petroleum station environments are sources of mixtures of hydrocarbon compounds resulting in enormous environmental pollution. This pose a serious hazard to petrol station attendants who work in that environment and are daily exposed to these hydrocarbon aerosols through inhalation during fuel dispensing, ingestion of contaminated edibles, as well as from gases emitted from vehicle exhaust [1]. Benzene, which has been established as a major content of petroleum is a contaminant that is widely distributed in the environment and several studies has associated it with hematological disorders including lowered hemoglobin and red blood cell values [5, 6, 7]. Short or long-term exposure to fumes from petroleum products that contain toxic chemicals such as benzene can heighten the risk of leukemia, anemia, thrombocytopenia loss of consciousness and convulsion. This exposure also has adverse effects on blood coagulation and fibrinolytic mechanisms. Notably, these toxic effects are attributed to the rapid absorption of benzene vapor into the blood and its distribution throughout the body following inhalation [2,8].

The serum iron test measures the level of iron in the blood whereas the total iron binding capacity (TIBC) is a measure of the quantity of iron that can bind to transferrin, a protein that serves as a transporter for iron. A high level of serum iron can signify iron overload disorders such as hemochromatosis or iron poisoning, whereas a low level of serum iron may suggest anemia associated with iron-deficiency or chronic disease. Conversely, a high TIBC level indicates a low amount of iron in the blood, which may be caused by iron-deficiency anemia, chronic infections, or other medical conditions and a low TIBC level, on the other hand, may be caused by iron overload disorders such as hemochromatosis, liver disease, or chronic transfusion therapy [2]. Exposure to petrochemical gas can lead to alterations in iron levels due to their potential to interfere with iron absorption or utilization in the body. The erythrocyte sedimentation rate (ESR) is a non-specific marker of inflammation and is elevated in the presence of inflammatory conditions which may include chronic exposure to petroleum products.

Several studies in the past focused on the impact of exposure to petroleum products on basic physiological indices such as full blood count but not on iron hemostasis and inflammation. This study therefore aims to fill this gap by determining changes in the levels of some iron indices and their association with the work-related duration of exposure to petroleum products by petrol station attendants.

Materials and Methodology

Study Setting

This study was conducted in Nnewi and Awka metropolis Anambra State, south-eastern Nigeria. They are both urban areas in Anambra state. Awka is the state capital and Nnewi is a major commercial center of the state. Both cities have numerous petrol stations that serve the large population of inhabitants. Each petrol station is serviced by several petrol station attendants.

Design of Study

This cross-sectional study was designed to measure levels of total iron binding capacity (TIBC), serum iron, and erythrocyte sedimentation rate (ESR) in petrol station attendants and compare them with those of control subjects.

Study Population

The study population included all petrol station attendants at fuel stations within the Nnewi and Awka metropolises in Anambra state.

Sampling Technique

A consecutive sampling method was applied in the recruitment of the participants in this study. Petrol station attendants who met the inclusion criteria were recruited consecutively from identified petrol stations until the sample size was attained.

Sample Size Calculation

G-power software (version 3.0.10) was used for sample size calculation. Power analysis for differences between two independent means was conducted. The calculated minimum sample size of 100 has 80% power to detect a medium effect size (difference of 0.40) at a significance level of 0.05.

Study Participants

A total of one hundred participants comprising 50 petrol station attendants (test participants) who were directly exposed to petroleum products in the course of their duties and 50 apparently healthy subjects who were not involved in activities that exposed them to petroleum products were recruited as the control (unexposed) group for this study. The petrol station attendants were aged 18 to 25 years and majority of them just completed their secondary school education and were either awaiting admission into tertiary institutions or seeking better employment opportunities. Few were undergraduate students that took up long vacation jobs to raise funds for their studies. The petrol station attendants worked a shift of 6 hours daily from (8am to 2pm and 2pm to 8pm). The petrol stations were all sited along major roads with high volume of traffic and the petrol stations did not differ in ventilation. The control subjects were undergraduate students of Nnamdi Azikiwe University who reside in the same city with the test subjects. They were age and gender-matched with the test subjects. They were not on any special diet and had similar socioeconomic background and environmental exposure as the test subjects. The major difference was that they were not residing close to fuel stations and were not exposed to petroleum products occupationally or habitually.

Inclusion Criteria

Healthy individuals that had worked as petrol station attendants for at least one month were included as test subjects, whereas apparently healthy undergraduate students not exposed to petrochemical gases were included as control subjects.

Exclusion Criteria

Individuals who were not petrol station attendants, Petrol station attendants that had worked for less than one month, female petrol station attendants and undergraduate students (controls) who were either pregnant or menstruating at the time of the study, individuals that were sick, those on iron supplements or other medications that affects iron, those diagnosed with any chronic infection or diseases, individuals that had blood transfusion in the previous three months prior to the study, smokers and habitual alcohol drinkers were excluded from the study.

Ethical Approval

Ethical approval for this study was gotten from the ethics committee of the Faculty of Health Sciences and Technology at Nnamdi Azikiwe University (FHST/REC/023/00244) in accordance with ethical principles for research involving human participants. The modalities for the study were thoroughly explained to all petrol station attendants at selected petrol stations and the controls. Each participant gave informed consent prior to their recruitment into study. The participants were given absolute liberty to decline or withdraw from participation with no consequences attached.

Sample collection and Laboratory analysis

Five milliliters (5ml) of blood was collected from each participant aseptically by venepuncture using a plastic syringe. Two milliliters (2ml) of the whole blood was added into commercially prepared ethylenediaminetetraacetic acid (EDTA) bottles. The samples were used immediately after collection to carry out erythrocyte sedimentation rate (ESR) test within an hour. The remaining three milliliters (3ml) of blood were dispensed into plain containers and allowed to clot. The clotted blood samples were centrifuged and the serum obtained was tested immediately for Iron and total iron binding capacity (TIBC).

Measurement of serum iron and total iron binding capacity (TIBC) levels

Serum iron and TIBC were measured by the Ferrozine (spectrophotometric) method using Iron/TIBC reagent set by Teco Diagnostics, USA. Tubes were labeled as "Test", "Standard", "Controls (low, normal and high controls)" and "Blank". Buffer (2.5ml) solution was added to the tubes. A total of 500µl of the serum, standard and control samples were dispensed into the respective tubes except the blank to which was added 500µl of iron free deionized water and mixed. The reagent blank (deionized water) was used to zero the spectrophotometer at 560nm. The absorbances of all the tubes were taken and recorded as A1. Afterward, 50µl of color reagent was dispensed into all the tubes and properly mixed. The tubes were then incubated at 37oC for 10 minutes. The spectrophotometer was zeroed at 560nm with the reagent blank. The absorbance of all the tubes was then taken and recorded as A2.

Calculations

Total Iron (ug/dL) = $\frac{A2\ Test - A1Test}{A2\ Std - A1\ Std} \times$ Concentration of Std

UIBC (ug/dL) = Concentration. of std − ($\frac{A2\ Test - A1Test}{A2\ Std - A1\ Std}$) × Concentration of Std

TIBC (ug/dL) = Total Iron + UIBC

UIBC = Unsaturated iron binding capacity

Std = Standard

Quality control procedures: The test kits have good assay precision with an intra-assay coefficient of variation of 2.1% which suggests good precision. Reagent samples with low, normal and high values were included as controls. The spectrophotometer used for the assay was properly calibrated before use. Standard and control samples were run together with the test samples to ensure reliability of readings obtained. Absence of haemolysis in the serum (test) samples was ensured.

Range of expected values:

Iron: 60-150µg/dL

TIBC: 250-450µg/dL

Measurement of erythrocyte sedimentation rate (ESR)

This was carried out by the Westergren method. The anticoagulated blood was mixed thoroughly with trisodium citrate at a ratio of 4:1. The blood-trisodium citrate mixture was drawn into the Westergren tube up to the zero-mark using pipette filler. The tubes were set upright in a vertical position and left undisturbed for 1 hour. At the end of 1 hour, the result was read and recorded.

Statistical Analysis

The data analysis was carried out using statistical package for social sciences (SPSS) version 25. A normality test was conducted to ascertain distribution of each variable (ESR, Iron and TIBC) using the Kolmogorov-Smirnov statistic The P-value (P > 0.05) and the shape of the histogram generated (pictorial distribution of data) indicated that the variables were normally distributed. Thus, parametric test statistics were chosen for analysis, and data were presented as mean ± SD in tables. The relationship between parameters was assessed using Pearson's correlation. The differences in mean values between parameters were assessed using independent t-test while the differences among parameters were assessed using one-way ANOVA. Post-hoc test using the Bonferroni correction for significant comparisons were applied for intergroup comparison. P values <0.05 were considered statistically significant.

Results

The mean values of serum iron and TIBC were significantly decreased in petrol station attendants compared to the control subjects (P = 0.028 and 0.048 respectively). The erythrocyte sedimentation rate did not differ significantly when compared between the two groups (see Table 1)

The ESR was significantly greater in petrol station attendants who had worked for > 12months than in those who had worked for 7 - 12 months and 1-6 months (P<0.05). Additionally, the serum iron and TIBC levels were significantly lower in petrol station attendants who had worked for >12months than in those who had worked for 1-6 months (P=0.033 and < 0.001 respectively) (see Table 2).

There was a significant weak-to-moderate positive correlation between the duration of work and the ESR (P= 0.005) and a significant weak-to-moderate negative correlation between the duration of work and TIBC (P = 0.013) (Table 3)

Among the petrol station attendants, the mean values of serum iron were significantly lower in females than in males whereas the ESR was greater in females than in males. Among the control subjects, the mean levels of serum iron and TIBC were greater in males than in females whereas ESR was lower in males than in females (P < 0.05) (see Table 4).

Table 1: Comparison of the mean serum iron concentration (µg/dL), ESR (mm/hr) and TIBC (µg/dL) between petrol station attendants and unexposed control subjects using independent t-test

Parameters	Petrol station attendants (n=50)	Unexposed control subjects (n=50)	P values	95% CI
ESR	19.30 ± 14.47	16.28 ± 12.31	0.264	[-2.31, 8.35]
Serum Iron	111.98 ± 58.55	143.82 ± 81.94	**0.028**	[-60.10, -3.58]
TIBC	382.06 ± 106.72	416.98 ± 136.62	**0.048**	[-83.57, -13.73]

P<0.05 = significant
ESR means Erythrocyte sedimentation rate
TIBC means Total iron binding capacity
µg/dL means microgram per deciliter
mm/hr means millimeter per hour
CI = Confidence interval

Table 2: Comparison of the mean iron concentration (µg/dL), TIBC (µg/dL) and ESR (mm/hr) among petrol station attendants based on the duration of employment using One-way ANOVA

Duration of employment	ESR	Serum Iron	TIBC
(A) 1-6 Months (n=27)	16.70 ± 13.56	117.83 ± 35.68	427.52 ± 106.75
(B) 7-12 Months (n=12)	15.33 ± 14.31	111.63 ± 68.01	364.33 ± 86.22
(C) >12 months (n=11)	30.00 ± 12.64	96.64 ± 55.10	289.82 ± 51.75
F(P) values	4.44 (0.017)	4.48 (0.022)	8.89 (0.001)
A vs B - P values	0.772	0.915	0.056
A vs C - P values	**0.009**	**0.033**	**<0.001**
B vs C - P values	**0.013**	0.395	0.061

P<0.05 = significant
ESR means Erythrocyte sedimentation rate
TIBC means Total iron binding capacity
µg/dL means microgram per decilitre
mm/hr means millimeter per hour

Table 3: Correlation of the mean iron concentration (µg/dL), TIBC (µg/dL) with duration of work and ESR (mm/hr) among petrol station attendants using Pearson correlation

Correlation	**r-values**	**P values**
ESR vs Serum Iron	-0.184	0.201
ESR vs TIBC	-0.200	0.163
Duration vs ESR	0.395	**0.005**
Duration vs Serum Iron	-0.115	0.425
Duration vs TIBC	-0.349	**0.013**

P<0.05 is significant
ESR means Erythrocyte sedimentation rate
TIBC means Total iron binding capacity
µg/dL means microgram per decilitre
mm/hr means millimeter per hour

Table 4: Comparison of mean serum iron concentration (µg/dL), TIBC (µg/dL) and ESR (mm/hr) among petrol station attendants and unexposed controls based on sex differences using independent t-test

Group	Parameters	Males	Females	t values	P values
Petrol station attendants	ESR	13.90 ± 11.37	27.40 ± 15.09	3.605	**0.001**
	Serum iron	125.97 ± 66.35	91.00 ± 36.75	2.143	**0.037**
	TIBC	391.17 ± 124.61	368.40 ± 73.08	0.736	0.466
Unexposed control subjects	ESR	12.17 ± 7.16	22.45 ± 15.68	3.145	**0.003**
	Serum Iron	166.43 ± 93.79	109.90 ± 43.25	2.517	**0.015**
	TIBC	481.87 ± 133.88	319.65 ± 64.51	5.031	**<0.001**

P<0.05 = significant
ESR means Erythrocyte sedimentation rate
TIBC means total iron binding capacity
μg/dL means microgram per decilitre
mm/hr means millimeter per hour

Discussion

This study assessed the changes in the levels of iron, total iron binding capacity and erythrocyte sedimentation rate in association with work-related duration of exposure to petrochemical products in petrol station attendants. Exposure to petrochemical gases occurs through inhalation or ingestion and has various effects on the human body, including possible changes in iron metabolism and inflammation.

This study revealed a significant decrease in the levels of serum iron in petrol station attendants compared to the level in control subjects. This finding seems to suggest that work-related exposure to petroleum products may be associated with reduced levels of serum iron than in unexposed individuals. While the validity of this association requires further research, other researchers have reported supporting and contrasting finding in this regard. Similar to our findings, Twaij et al [9] and Abdel et al [10] reported a significant decrease in iron levels of the petrol station workers in their own research. However, on the contrary, Alhadithi and Kadhim [11] discovered a significant increase in the mean levels of iron among fuel workers compared to their control participants. Additionally, in a study by Airhomwanbor et al [12] conducted in Ekpoma Nigeria that assessed some heavy metals including iron levels in petrol station attendants. They reported absence of any significant difference in iron levels in petrol station attendants compared with controls. Theoretically, iron is a vital content of hemoglobin found in red cells thus, a decrease in iron levels can result in anemia if not properly managed. When anemia results from a decrease in serum iron as observed in our study it may suggest a predisposition to iron deficiency anemia. In exploring the link between exposure to petroleum products and anemia, Lin et al[13] reported that among gasoline exposed workers, the prevalence of anemia was significantly greater than among non-exposed groups. Hypothetically, the possible mechanism by which exposure to petroleum products cause a change in iron indices and predispose to anemia varies. On one hand it is proposed that petroleum products generate reactive oxygen species that induce oxidative stress that leads to an imbalance in oxidant - antioxidant levels in the body [14, 15]. These reactive oxygen species have capacity to oxidize the iron located inside the haem moiety of the hemoglobin molecule in red blood cells. This culminates in the change of iron from a ferrous to a ferric state and loss of its ability to play its normal role of binding and transporting oxygen molecules [16]. On the other hand, it's hypothesized that long-term exposure to petroleum products can lead to suppression of the blood cell production function of the bone marrow leading to reduction in production of red blood cells [17, 18, 19]. Additionally, genotoxic potentials of benzene which is a constituent of petroleum products have been hypothesized. It is proposed that based on this potential, it can cause DNA damage and alteration in the expression of genes linked to red cell production which may lead to anemia [20].

This study revealed a significant decrease in the serum levels of TIBC of petrol station attendants compared to the control subjects. Since TIBC measures the capacity of transferrin (a protein that transports iron in the blood) to bind iron, this finding suggests a reduction in the capacity of transferrin to bind and transport iron in individuals exposed to petroleum products in relation to control subjects. The erythrocyte sedimentation rate is a non-specific marker of inflammation; thus, elevated ESR values may sometimes be suggestive of an inflammatory condition. This research reported no significant change in erythrocyte sedimentation rate of petrol station attendants compared with control individuals who were not exposed to petrochemical gas. This finding do not align with a previous study by Jabbar and Ali [17] that reported a significantly higher erythrocyte sedimentation rate in workers exposed to petroleum products than in non-exposed workers.

Previous studies have reported that chronic exposure to petrochemical gases increases the risk of toxicity [21, 22]. In this study self-reported duration of employment was assessed as a measure of duration of exposure. The idea is that those who have worked for a longer time will be more exposed than those who had worked for a shorter period. While this may seem justified hypothetically, it has its limitations. A direct measurement of actual exposure to petroleum products would have been a more accurate and objective assessment of exposure. The present study found that work-related duration of exposure to petrochemical products results in some changes in the assessed parameters. A significant reduction in serum iron and TIBC levels were observed in petrol station attendants who had worked for >12months compared with those who had worked for 1-6 months. This aligns with the significant weak-to-moderate negative relationship observed between work-related duration of exposure and level of TIBC. This suggests that the level of TIBC decreases with increasing duration of work-related exposure and vice versa. However, there was no significant correlation between work-related duration of exposure and level of iron in this study. This agrees with the findings of Alhadithi and Kadhim [11] and Twaij et al [9] that also reported that there was no significant relationship between years of exposure and iron levels in fuel workers. The erythrocyte sedimentation rate was significantly greater in petrol station attendants who had worked for > 12 months than in those who had worked for a shorter duration of 7-12 months and 1-6 months. This finding also supports the significant but weak-to-moderate positive relationship between the duration of exposure and ESR. These findings suggest that ESR values increase with longer work-related duration of exposure to petrochemical products among attendants at petrol stations.

The research also revealed significant sex-related differences in the parameters measured. Among the petrol station attendants, the serum iron level was significantly greater in males than in females; however, among the controls, both the serum iron level and TIBC were higher in males than in females. The ESR was lesser in males than in females for both the petrol station attendants and the control participants. These findings may be suggestive of sex-dependent variations in these parameters.

In view of the findings of this study, it is worrisome that all the petrol station attendants sampled in this study did not use facemasks or face shields, while only a few used protective work clothing. Therefore, the finding of the present study highlight the necessity for owners of petrol stations to prioritize safety regulations and ensure the enforcement of occupational safety measures including the use of protective gadgets such as face masks and protective wears or work clothes. They also need to put measures in place to improve ventilation, minimize the concentration of petroleum product fumes in the work environment and provide occupational safety education to staff members to minimize exposure to the risks associated with petrochemical gases.

However, this study has some limitations. Firstly, it was a cross-sectional study that has a limited ability to measure time-dependent changes; thus, a longitudinal study that involves a follow-up measurement for each participant is recommended in future studies to ascertain the impact of cumulative exposure. Also, self-reported work-related duration utilized in this study has its limitation and may negatively affect dose-response interpretation. Personal exposure monitoring device is suggested in future studies to fill this gap. Additionally, other more specific markers of inflammation need to be measured to establish accurately the contribution of inflammation to the changes observed in the serum iron and TIBC levels. Future studies need to investigate the contributory toxicity of the various constituents of petroleum and the interaction between each component and how it influences the changes observed in iron parameters. Finally, this study did not factor in the nutritional history and dietary information of the participants which could affect the levels of iron parameters.

Conclusion

This study revealed a possibility that work-related exposure to petrochemical gases may be associated with reduction in the serum levels of iron and levels of TIBC. This highlights the need for improved periodic occupational health assessment and the enforcement of the use of personal protective equipment by petrol station workers to minimize the inhalation of petroleum products.

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